

SO₂ POLLUTION, I. INFLUENCE OF ACIDIFICATION ON BACTERIAL COMMUNITIES OF FOREST SOILS

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Abstract

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The increasing stress of spruce-forest soils by SO₂ immissions causes a decrease in the numbers of heterotrophic bacteria in the fermentative horizon and an increase in the numbers in the mineral horizon A. Horizon A acidifies progressively. Bacterial communities of the fermentative horizon from various types of forest stands (spruce forest without ground vegetation, grassy mountain ash) differed by one order at a similar soil pH. In acid forest soil, acidophilic bacteria predominated over neutrophilic bacteria. The biochemical activities of both groups are very similar. The outfit of bacteria with biochemical activities is influenced rather by the nutritional conditions of the environment than by its pH. Acidification of the environment, if present, is not sufficient for explanation of the changes in the bacterial communities observed in the exposed areas.

The development of energetics based on the burning of less valuable coal causes sulphur dioxide to escape into the atmosphere. This phenomenon is connected with the occurrence of acid rain (Brosset, 1973). Literature, mainly that of popular character (Pearce, 1982), draws attention urgently to the ecological hazard of acid rain. Under the stress of negative ecological changes in northern lakes, caused by the decrease of pH of the poorly buffered water, the acidification of the environment is considered to be a very serious phenomenon (for review see Wright, 1976). The negative influence of industrial immissions in terrestrial ecosystems is manifested especially in forests, as large areas of forests are exposed to the long-term influence of immissions. It is impossible to affect forest soil to such an extent as in agriculture. Damage to forest is often explained as being caused by acidification (Jonsson, 1976). In polluted areas, impaired soil microflora was observed (Langkramer, 1974; Mrkva and Grunda, 1969; Langkramer and Lettl, 1982), which is considered to be connected with the destabilization of the whole forest ecosystem caused by acid rain (Ulrich, 1982).

It was shown that mainly the bacterial component of the soil microbial community is sensitive to the influence of the immissions. Therefore, the aim of this paper is to ascertain the influence of soil acidity on bacterial communities of forest soil, mainly of spruce forests.

Materials and methods

The experimental areas were chosen in NW Bohemia in Slavkov Forest and Ore Mountain. In Slavkov Forest, spruce forests near the villages Kladská (870 m above sea level) and Trstěnice (540 m) were selected as relatively non-polluted areas. A forest near the village Prameny (835 m) was taken as a slightly stressed area. Spruce forests near Šabina (490 m) and Hrušková (620 m) are strongly exposed to SO₂ immissions, moreover the latter to the fall-off of fly-ash.

In Ore Mountain, near the village Nová Ves, the spruce forests were almost completely destroyed. At an altitude of 730 m, a remnant of a young spruce growth without ground vegetation, a withered developed spruce forest with a strongly developed grass undergrowth (mostly *Calamagrostis villosa*) and a mixed grassy growth of mountain ash and birch, succeeding after the devastated spruce forest, have been chosen for the experiment. Detailed characteristics of the areas were published elsewhere (Langkramer and Lettl, 1982).

Soil sampling: the samples from fermentative and humus horizons and from horizon A were taken separately. Physical, chemical and biochemical analyses of soil samples were carried out according to Klika et al. (1954) and Schlichting and Blume (1966) using 2 mm soil.

The numbers of aerobic bacteria were determined on Thornton's agar, the numbers of ammonifying bacteria on meat-peptone agar prepared from Nutrient broth No.2 (both media purchased from Imuna, Šarišské Michalany, Czechoslovakia), after 4 days of incubation at 25 °C. If not stated otherwise, the pH of media were left at the value given by the producer (pH 7.0 ± 0.2).

The same media were used for the isolation of bacterial cultures. The biochemical activities of pure cultures were tested in the same media, too (liquid or solid), if not stated otherwise. The ability to attack cellulose was proved by using Schleicher-Schuell paper No.589 (incubation 8 weeks), the amylolysis was screened on agar containing 1 % Starch Soluble (Lachema, Czechoslovakia), proteolysis was proved by liquefaction of medium containing 6 % Bacto-Gelatin (Difco). The ammonification of asparagine was tested in liquid Thornton's medium; the NH₄⁺ produced was proved by means of the Nessler reagent. The oxidation of NH₄⁺ and NO₂⁻ was determined in a solution of inorganic salts (Pochon, 1954) at pH 4.0, 6.0 and 8.0. The produced NO₂⁻ was evaluated after 3 weeks' incubation using a reaction with sodium salicylate (Hofmann et al., 1965). The ability of the isolates to reduce elemental sulphur to S²⁻ was tested in liquid media with 30 mmol/L S⁰ and 1 mmol/L FeSO₄ (control without S⁰). For the oxidative reactions, media with 30 mmol/L S⁰ or 5 mmol/L Na₂S₂O₃ were used. In the culture supernatants (6.000 g, 10 min), ions SO₄²⁻ were sought with 0.2 M BaCl₂. The duration of the incubation period was 1 week, if not stated otherwise. Thiosulphate sulphurtransferase (rhodanese; EC 2.8.1.1.) was determined by the method described elsewhere (Lettl, 1983). Thiosulphate oxidase and sulphite oxidase (EC 1.8.3.1) were determined in 3 day-old cultures from the decrease of absorbance (A_{420 nm}) of the solution of K₃[Fe(CN)₆] (Hall and Berk, 1972) under aerobic conditions in the mixture of 1 ml of the culture supernatant, 3 ml 50 mM Tris-H₂SO₄ buffer pH 6.0, 0.5 ml 0.66 M Na₂S₂O₃ or Na₂SO₃ and 0.5 ml 0.1 M K₃[Fe(CN)₆] in the same buffer. Supernatants inactivated by boiling for 30 minutes and a noninoculated medium served as controls.

Results

Soil acidity

During the years 1971 to 1979, the influence of SO₂ immissions on the soil microbial community in the areas in the region of Slavkov Forest was studied. The

Table 1. Average values ($\bar{x} \pm S_x(n)$) of soil pH, b value from regression equations of the soil pH development, concentrations of aerobic bacteria (in thousands per g oven dry soil) and intensity of ammonification (in mg $\text{NH}_4\text{-N}$ released from 1 kg soil in 14 d) in the soils from five localities in Slavkov Forest (Langkramer and Lettl, 1982)

Elevation above sea level in m	Horizon	Locality	Its character	pH (H ₂ O)	b value	Aerobic bacteria	Ammonification
500—600	F	Trstěnice	pure	3.82 ± 0.21 (24)	-0.000 124	$3\,683 \pm 6\,606$ (13)	147 ± 133 (19)
		Šabina	heavy polluted	3.86 ± 0.18 (24)	0.000 475	$2\,537 \pm 6\,449$ (14)	79.1 ± 81.6 (19)
		Hrušková	heavy polluted	3.78 ± 0.34 (28)	-0.001 182	$1\,450 \pm 1\,848$ (19)	49.2 ± 82.7 (23)
	H	Trstěnice	pure	3.49 ± 0.12 (23)	0.000 925	243 ± 245 (13)	79.8 ± 77.2 (19)
		Šabina	heavy polluted	3.49 ± 0.16 (24)	0.000 602	255 ± 339 (12)	7.7 ± 42.3 (19)
		Hrušková	heavy polluted	3.58 ± 0.38 (28)	0.000 948	266 ± 283 (18)	20.4 ± 47.4 (23)
	A	Trstěnice	pure	3.52 ± 0.18 (16)	0.000 014	65.9 ± 117 (11)	12.5 ± 23.2 (17)
		Šabina	heavy polluted	3.55 ± 0.12 (16)	-0.005 780	108 ± 150 (10)	6.4 ± 10.5 (17)
		Hrušková	heavy polluted	3.55 ± 0.44 (20)	0.000 594	194 ± 221 (16)	5.0 ± 8.0 (21)
800—900	F	Kladská Prameny	pure	3.70 ± 0.30 (26)	0.000 424	$888 \pm 1\,444$ (18)	115 ± 96.0 (22)
			slightly polluted	3.56 ± 0.81 (26)	0.000 218	730 ± 915 (15)	89.8 ± 97.2 (21)
	H	Kladská Prameny	pure	3.48 ± 0.27 (26)	0.000 858	250 ± 300 (17)	33.1 ± 37.4 (22)
			slightly polluted	3.47 ± 0.38 (26)	0.000 230	439 ± 711 (16)	17.1 ± 46.7 (21)
	A	Kladská Prameny	pure	3.50 ± 0.34 (18)	0.000 603	187 ± 200 (17)	6.4 ± 18.7 (20)
			slightly polluted	3.45 ± 0.44 (18)	-0.001 825	234 ± 301 (16)	17.4 ± 17.5 (19)

Table 2. Soil pH(H₂O) and numbers of bacteria (in thousands per g oven dry soil) in soil horizons from three types of forest stands (Ore Mountains, locality Nová Ves)

Horizon	Date of sampling	Soil pH(H ₂ O)			Aerobic bacteria			Ammonifying bacteria		
		spruce	dead spruce	mountain ash	spruce	dead spruce	mountain ash	spruce	dead spruce	mountain ash
F	Jun 3, 1982	3.10	3.00	3.50	859	7 741	3 515	1 970	10 661	6 540
	Oct 5,	3.55	3.45	3.95	1 643	2 763	6 440	1 317	1 605	4 853
	Apr 18, 1983	3.40	3.90	4.00	612	20 462	30 803	1 145	44 825	45 324
	Jun 23,	4.10	4.40	4.40	3 238	12 867	34 636	978	7 364	32 535
	Aug 9	3.00	3.90	3.90	281	476 474	37 254	281	466 531	36 134
H	Jun 3, 1982	3.20	2.90	3.00	228	1 038	470	488	645	677
	Oct 5,	3.20	3.50	3.70	1 590	1 923	5 200	210	890	3 320
	Apr 18, 1983	3.60	3.50	3.50	528	1 125	2 345	603	1 239	1 961
	Jun 23,	3.60	3.70	3.90	2 636	5 338	15 106	303	6 920	13 842
	Aug 9,	3.10	3.30	3.30	312	1 554	1 822	312	1 170	786
A	Jun 3, 1982	2.90	3.10	3.10	36.9	62.0	37.8	79.0	300	99.1
	Oct 5,	3.15	3.40	3.65	1 217	1 493	3 440	173	227	83.3
	Apr 18, 1983	3.40	3.40	3.30	196	110	263	156	281	379
	Jun 23,	3.70	3.80	3.70	1 946	3 801	2 657	50.3	433	199
	Aug 9,	3.20	3.40	3.50	205	294	174	206	86.7	275

Table 3. Optimum pH for the growth of bacterial populations

Elevation above sea level in m	Locality; pH(H ₂ O) of the soil sample	pH of the media	Per cent of colonies on the agar medium	
			Thornton	meat-peptone
500—600	Trstěnice pH 3.7	4.0	90.1	0.7
		5.0	95.3	6.2
		6.0	100.0	21.3
		7.0	57.3	51.1
		8.0	34.2	100.0
		9.0	24.4	1.8
	Šabina pH 3.4	4.0	100.0	0.89
		5.0	82.3	20.0
		6.0	62.8	32.7
		7.0	45.5	72.7
		8.0	41.6	100.0
		9.0	31.6	18.6
700—900	Kladská pH 3.1	4.0	100.0	3.6
		5.0	84.6	21.4
		6.0	58.9	94.6
		7.0	29.4	100.0
		8.0	15.7	37.5
		9.0	14.5	18.7
	Nová Ves pH 3.0	4.0	100.0	2.3
		5.0	62.0	17.8
		6.0	56.1	100.0
		7.0	30.8	55.0
		8.0	25.6	23.2
		9.0	20.4	20.9

average soil pH values measured during the period are presented in Table 1 (Langkramer and Lettl, 1982).

The time course of soil pH values was estimated using the method of least squares (Langkramer and Lettl, 1982). Table 1 presents the value *b* from regression equations characterizing the slope of the lines.

The bacterial community and the soil pH

The average concentrations of aerobic bacteria during the 9 year observation of areas in Slavkov Forest are compared together with the average soil pH in Table 1.

Recently, an observation of microbial colonization in Ore Mountain near the village Nová Ves is being carried out. The soil pH values from 3 types of stands

(young spruce forest, a withered spruce forest with grass undergrowth and a grassy mountain ash) are very similar. The data about the microbial colonization of soils available up to date are presented together with soil pH in Table 2.

Optimum pH for the growth of soil microbial communities

An optimum pH for the development of bacterial populations was studied by means of inoculation of soil suspensions on Thornton's and meat-peptone agar at pH values in the range of 4.0 to 9.0. We used samples from the fermentative horizons collected on January 12, 1983 in spruce forests in pure and polluted areas at altitudes of approximately 500 m above sea level (Trstěnice, pH of the soil sample 3.7; Šabina, pH 3.4) and at an altitude of approximately 800 m (Kladská, pH 3.1; Nová Ves, pH 3.0). The results are summarized in Table 3. The numbers of developed colonies are expressed as a percentage of the highest ascertained number.

Biochemical activities of bacteria from spruce forests

From the formerly described soil samples, altogether 200 cultures of heterotrophic bacteria were isolated on Thornton's (pH 4.0) and meat-peptone agar (pH 6.0 and 8.0 according to the found optima). Altogether 8 activities from the carbon, nitrogen and sulphur cycles were tested in the isolates. The results are summarized in Table 4 and are expressed as a percentage of active cultures in individual groups of cultures.

The nitrifying activity of the isolates was determined quantitatively (at the same pH values, which were used for the isolation of the cultures) from the amount of produced NO_3^- . Table 5 shows the percentual distribution of the isolates according to the amount of produced NO_3^- .

Biochemical activities of bacteria from various types of growth

Soils from the fermentative horizon were sampled in three types of forest stands on October 5, 1982 near the village Nová Ves (young spruce forest, grassy withered spruce forest and grassy mountain ash), the pH of which were very similar. Altogether 74 cultures of heterotrophic bacteria were isolated on meat-peptone agar. 12 biochemical activities were tested in these isolates (Table 6). The production of enzymes significant in the sulphur cycle was determined quantitatively. The active strains were divided into groups according to the intensity of enzyme production (Table 7). The distribution of the cultures according to the number of various activities which they possess, is shown in Table 8.

Elevation above sea level in m	Medium and pH for the isolation ^a	Locality	Its character	Total number of isolates	Per cent of isolates with the activity							
					cellu- lolytic	amylolytic	gelatino- lytic	ammoni- fication of aspara- gine	oxida- tion of NH ₄ ⁺ to NO ₃ ⁻	reduc- tion of S ⁰ to S ²⁻	oxida- tion of S ⁰ to SO ₄ ²⁻	oxida- tion S ₂ O ₃ ²⁻ to SO ₄ ²⁻
500—600	THA pH 4.0	Trstěnice Šabina	pure polluted	27 23	3.7 0	18.5 0	3.7 30.4	100.0 95.6	100.0 91.3	0 0	29.6 68.2	33.3 0
	MPA pH 8.0	Trstěnice Šabina	pure polluted	20 25	15.0 36.0	25.0 28.0	30.0 40.0	100.0 84.0	95.0 68.0	50.0 48.0	45.0 80.0	35.0 16.0
700—900	THA pH 4.0	Kladská Nová Ves	pure polluted	27 27	88.9 81.5	11.1 7.4	0 0	92.6 88.9	100.0 85.7	0 8.7	24.0 59.2	11.1 29.6
	MPA pH 6.0	Kladská Nová Ves	pure polluted	26 25	84.6 92.0	69.2 36.0	3.8 0	92.3 96.0	80.8 64.0	38.5 4.0	52.2 88.0	0 0

Table 5. Effectivity of bacterial isolates in nitrate production in vitro

Elevation above sea level in m	Medium and pH for the isolation	Locality	Its character	Total number of isolates	Per cent of isolates producing nitrate in the concentration (mmol . L ⁻¹) in 3 weeks							
					0	0.1—0.5	0.5—1.0	1.0—2.0	2.0—3.0	3.0—5.0	5.0—7.0	7.0
500—600	THA pH 4.0	Trstěnice Šabina	pure polluted	27 23	0 8.7	11.1 30.4	22.2 21.7	33.3 17.4	7.4 4.3	3.7 0	18.5 8.7	3.7 8.7
	MPA pH 8.0	Trstěnice Šabina	pure polluted	20 25	5.0 32.0	25.0 40.0	40.0 12.0	25.0 16.0	5.0 0	0 0	0 0	0 0
700—900	THA pH 4.0	Kladská Nová Ves	pure polluted	27 27	0 33.3	0 29.6	0 29.6	44.4 7.4	33.3 0	22.2 0	0 0	0 0
	MPA pH 6.0	Kladská Nová Ves	pure polluted	26 25	19.2 36.0	42.3 40.0	23.1 16.0	11.5 4.0	3.9 4.0	0 0	0 0	0 0

Discussion

Acid rain is considered to be a most significant agent causing the damage of forest stands and soil by immissions (Ulrich, 1982). However, direct proof of this is very difficult to obtain and the situation is evaluated carefully: "We have found no good reason for attributing the reduction in growth to any cause other than acidification" (Jonsson, 1976).

It is necessary to distinguish between at least two mechanisms involved in the influence of acidification on the soil microbial community: during the acute effect, the low pH of the environment may not be favourable for the existence and/or the activity of microorganisms. During a long-term exposure various secondary changes can take place (e.g. washing-out of cations, loss of nutrients, decrease in the ion exchange capacity of soils, mobilization of aluminium, etc.) (McFee et al., 1976; Lochman, 1981). Soil can then become a less favourable environment for microorganisms regardless to the actual pH value.

The experimental procedures generally employed deal mainly with the study of the acute effect during artificial acidification of soil: the numbers of heterotrophic bacteria, even by strongly acid watering, are lowered relatively insignificantly (Bååth et al., 1979; Bryant et al., 1979). Soil respiration is markedly inhibited only by using water of pH 2.0 but not of pH 3.0 (Bååth et al., 1979); the same refers to degradation of organic matter (Abrahamsen et al., 1976; Tamm et al., 1977) and the activity of soil enzymes (Killham et al., 1983). Artificial acidification decreased the nitrogen immobilization and nitrification (Tamm et al., 1977); the latter activity is considered especially sensitive to acidification. The production of infectious nodules on roots of leguminous plants by symbiotic nitrogen fixing bacteria is restricted (Shriner, 1977).

Thus, in experiments studying the acute influence of low pH, it was necessary to use very drastic acidification under pH 3.0 to obtain distinct effects on the microbial community. These experiments are not as meaningful for studies of the chronic influence of acidification: it was critically suggested that it would be essential to apply acid watering at least 15 years (Ulrich, 1982). In this connection even the mentioned 5-year experiment (Bååth et al., 1979) has a limited validity.

The question arises whether acid rain can achieve the pH values comparable with artificial acidification. In the studied region of Slavkov Forest the pH of rain-water was within the range of 3.6 to 5.8 on the free area. Rain—water, after passing through the spruce tops, acidified to pH values between 3.2 to 5.8 (Lochman et al., 1981). However, the fall-off was collected and measured at 1 month intervals, therefore it is possible that the pH of the rain could occasionally drop to 2.0. This decrease would probably evoke only a temporary change in the bacterial community.

In the average pH values of soils over the studied areas in Slavkov Forest there

Table 6. Occurrence of biochemical activities in bacterial isolates from soils of three types of forest stands

Activity	Numer (n) and per cent of active cultures		
	spruce n [%]	withered spruce n [%]	mountain ash n [%]
Cellulolysis	4 (16.7)	15 (68.2)	22 (78.6)
Amylolysis	9 (37.5)	13 (59.1)	27 (96.4)
Gelatinolysis	2 (8.3)	10 (45.4)	27 (96.4)
Ammonification of asparagine	15 (62.5)	19 (86.4)	28 (100.0)
Oxidation of NH_4^+ to NO_3^-	0	3 (13.6)	18 (64.3)
Oxidation of NO_2^- to NO_3^-	14 (58.3)	18 (81.8)	20 (71.4)
Reduction of S^0 to S^{2-}	7 (29.2)	12 (54.5)	26 (92.9)
Oxidation of S^0 to SO_4^{2-}	22 (91.7)	18 (81.8)	28 (100.0)
Oxidation of $\text{S}_2\text{O}_3^{2-}$ to SO_4^{2-}	6 (25.0)	12 (54.5)	28 (100.0)
Production of: thiosulfate sulfurtransferase (rhodanese)	2 (8.3)	10 (45.4)	26 (92.9)
thiosulfate oxidase	7 (29.2)	13 (59.1)	18 (64.3)
sulfite oxidase	2 (8.3)	3 (13.6)	7 (25.0)
Total number of isolates	24	22	28

are no substantial differences which could be attributed to the influence of immissions. Observations of the tendency of pH changes during the 9 years of the experiment proved that in the exposed areas only mineral horizons A are acidified somewhat faster, whilst the pH of organic horizons (the fermentative and humus horizons) oscillates around stable values (Table 1) (Langkramer and Lettl, 1982; Lochman et al., 1981). However, a most pronounced decrease of the concentration of heterotrophic bacteria is observed in the fermentative horizon. In the humus horizon, the influence of immissions is not clearly manifested. In the mineral horizon A, i.e. in the only one which acidifies gradually — the concentrations of bacteria even increase on the exposed areas.

On the contrary to the concentrations of bacteria, the soil activities (respiration, ammonification) were reduced considerably in the localities of Slavkov Forest to the depth of the mineral horizon A (Langkramer and Lettl, 1982). Thus, even biochemical activities are not determined by soil pH (Table 1).

Another possibility of the evaluation of pH significance is offered by the experimental areas near Nová Ves in Ore Mountain. Three types of growths (spruce forest without undergrowth, grassy withered spruce forest and grassy mountain ash stand) are located near each other and the pH of their soils are very similar. Nevertheless, soils of the areas with grass contained concentrations of bacteria higher by one order than the soil of the spruce forest without undergrowth (Table 2). Nitrification, often considered as an indicator of favourable soil

Table 7. Intensity of enzymes production in bacterial isolates from three types of forest stands

Enzyme concentration (nkat . L ⁻¹)	Number (n) and per cent of isolates from the forest stand		
	spruce n [%]	dead spruce n [%]	mountain ash n [%]
Thiosulphate sulphurtransferase (rhodanese)			
0—1	1 (4.2)	5 (22.7)	2 (7.1)
>1—5	—	2 (9.1)	8 (28.6)
>5—10	1 (4.2)	3 (13.6)	11 (39.3)
>10—15	—	—	3 (10.7)
>15—20	—	—	2 (7.1)
Thiosulphate oxidase			
100—200	—	—	1 (3.6)
>200—500	1 (4.2)	5 (22.7)	1 (3.6)
>500—1000	2 (8.3)	4 (18.2)	5 (17.8)
>1000—2000	2 (9.3)	3 (13.6)	2 (7.1)
>2000	2 (8.3)	1 (4.5)	9 (32.1)
Sulphite oxidase			
100—200	1 (4.2)	1 (4.5)	1 (3.6)
>200—500	—	1 (4.5)	1 (3.6)
>500—1000	1 (4.2)	—	3 (10.7)
>1000	—	1 (4.5)	2 (7.1)
Total number of isolates	24	22	28

conditions, was observed in these grassy stands. Changes in the bacterial colonization of soils were therefore probably evoked mainly by the rhizosphere effect of grass: the secretion of nutrients by roots in the vegetation season can be great (Přikryl and Vančura, 1980) and thus can overcome the pH influence.

The bacterial community is capable of adapting to the decreased pH of the environment. During the determination of the optimum pH for the growth of two nutritional bacterial groups from soils of various acidity, it was shown that the decrease of soil pH reflected in a decrease of pH optimum for aerobic bacteria (Thornton's agar) from 6.0 to 4.0 or less (which is impossible to prove experimentally due to acid hydrolysis of agar), for ammonifying bacteria (meat-peptone agar) from pH 8.0 to 6.0. Thus, it is evident that the number of neutrophilic bacteria decreased and acidotolerant or acidophilic bacteria become significant (Table 3).

These changes need not represent a deprivation of the bacterial community of biochemical activities. The summary of the main mineralization activities of the isolated pure cultures (Table 4) does not signalize any substantial deprivation with acidophilic bacteria isolated on Thornton's agar at pH 4.0 and tested at the same pH. They practically lacked only the ability to reduce S⁰ to S²⁻. Rather other

Table 8. Numbers of biochemical activities* found in bacterial isolates from F horizon of three types of forest stands

Number of activities	Number (n) and per cent of bacterial isolates from the soil of forest stand		
	spruce n [%]	withered spruce n [%]	mountain ash n [%]
1	1 (4.2)	—	—
2	5 (20.8)	—	—
3	8 (33.4)	1 (4.5)	—
4	2 (8.3)	2 (9.1)	—
5	3 (12.5)	3 (13.6)	1 (3.6)
6	2 (8.3)	5 (22.7)	—
7	3 (12.5)	2 (9.1)	—
8	—	6 (27.4)	3 (10.7)
9	—	2 (9.1)	6 (21.4)
10	—	1 (4.5)	8 (28.6)
11	—	—	8 (28.6)
12	—	—	2 (7.1)
Total number of isolates	24	22	28

* For a list of activities tested see Table 6.

influences are manifested: the altitude of the localities influenced the occurrence of cellulolytic, amylolytic and proteolytic activities. Pollution of the areas by sulphur caused a markedly increased incidence of the ability of heterotrophic bacteria to oxidize S^0 to SO_4^{2-} . The nitrifying activity was observed almost in all isolates. The acidophilic cultures isolated and tested at pH 4.0, exhibited significantly higher effectivity in NO_3^- production than isolates from meat-peptone agar with pH near to neutral. The production of nitrates was generally lower in isolates from the polluted areas (Table 5) even after 4 passages on standard media, so that the direct influence of immissions was excluded. With regard to the acidophilicity of the isolates it would not be reasonable to explain their permanent damage by the effect of the low pH of the environment.

Table 6 presenting a survey of activities of bacteria from the three types of growth with similar soil pH confirms that the occurrence of the activities cannot be attributed to soil pH. Moreover, it was shown that the nutritional effects are much more pronounced. The occurrence of activities in heterotrophic bacteria rapidly increased also (Table 7). Therefore, bacterial strains from the mountain ash forest stand are supplied with a substantially higher number of biochemical activities than isolates from spruce forest soil: in the spruce soils most bacteria reflected only 2 or 3 activities, whereas in mountain ash stand, the highest percentage of bacteria reflected 10 or 11 activities from the 12 tested (Table 8).

Acidification represents a factor that cannot be ignored: the change of pH by liming always improved the microbial conditions in polluted soils (Król et al., 1972). However, from the presented results, a possibility arises, that soil pH and gradual acidification of the soil in the studied range does not represent a decisive factor of the immission influences on soil bacterial communities. Also it is probably impossible to attribute deep changes in soil biochemical activities to the acidification process alone.

Translation submitted by the author

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Lettl A.: Imise SO₂. I. Vliv acidifikace na bakteriální společenstva lesních půd.

Vzrůstající zatížení půd smrkových porostů imisemi SO₂ má za následek depresi počtu heterotrofních bakterií ve fermentačním horizontu F a zvýšení počtů v minerálním horizontu A. Horizont A se progresivně okyseluje. Bakteriální osídlení fermentačního horizontu z různých typů porostů (smrkový porost bez přizemní vegetace, zatrávněný porost jeřábu) se liší řádově při velmi podobném půdním pH. V kyselých lesních půdách převládají bakterie acidofilní nad neutrofilními. Biochemické aktivity obou skupin jsou velmi podobné. Výbava bakterií biochemickými aktivitami je ovlivněna spíše nutričními podmínkami prostředí než jeho pH. Acidifikace prostředí (je-li jaká) nestačí k vysvětlení změn v bakteriálních společenstvech, pozorovaných v zatížených oblastech.

Леттл А.: Имисии SO₂. I. Влияние подкисления на бактериальные сообщества лесных почв.

Возрастающее загрязнение почв еловых насаждений имисиями имеет в своем последствии понижение количества гетеротрофных бактерий в ферментативном горизонте и повышение количества в минеральном горизонте А. Горизонт А прогрессивно подкисляется. Бактериальные заселения ферментативного горизонта из разных типов насаждений (еловые насаждения без подлеска, травянистое насаждение рябины) отличались на порядок при подобном почвенном pH. В кислых лесных почвах преобладают бактерии ацидофильные над нейтрофильными. Биохимические активности обеих групп являются очень похожими. Набор биохимических активностей у бактерий находится под влиянием скорее условий питания среды чем ее pH. Подкисление среды (если есть какое нибудь) не хватает к объяснению изменений в почвенных бактериальных сообществах, исследованных в загрязненных областях.